

THE GENETIC RELATIONSHIP BETWEEN  
*BOMBUS FRANKLINI* (FRISON) AND  
OTHER TAXA OF THE  
SUBGENUS *BOMBUS* S.STR. (HYMENOPTERA: APIDAE)

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*Abstract.*—*Bombus franklini* (Frison) has either been regarded as a distinct species or has been synonymized with *B. occidentalis* Greene. We surveyed 21 enzymes by vertical starch-gel electrophoresis and compared *B. franklini* with *B. occidentalis* and nine other species of the subgenus *Bombus* sensu stricto. We found that *B. franklini* differs from *B. occidentalis* at three enzyme loci and there was no evidence of intergradation in areas of sympatry. According to the electrophoretic data *B. franklini* is close to two species groups which comprise: (a) *B. cryptarum* (Fabr.), *B. magnus* Vogt, *B. moderatus* Cresson and *B. hypocrita* Pérez; and (b) *B. occidentalis*, *B. terricola* Kirby and *B. lucorum* (L.), while *B. terrestris* auct., *B. affinis* Cresson and *B. sporadicus* Nylander are more distant.

*Key Words.*—Insecta, Apidae, enzyme electrophoresis, bumble bee genetic relationships

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Among the North American bumble bee species, *Bombus franklini* (Frison) has by far the smallest area of geographical distribution. All recent records have been taken within a 60 mile radius of Grants Pass, Oregon (Thorp 1970). *Bombus franklini* has puzzled entomologists for a long while. It was described from the Oslar collection (Frison 1921) and the holotype was apparently erroneously recorded from Nogales, Arizona, as discussed by Thorp (1970), who proposed Gold Hill, Jackson County, Oregon, as the new type locality. Milliron (1971) considered *B. franklini* conspecific with *B. occidentalis* Greene. Thorp et al. (1983), however, had collected *B. franklini* at several localities sympatrically with *B. occidentalis* and did not find intergrades between them. Plowright & Stephen (1980), working on a multivariate analysis of wing venation data taken from queens, were able to show a clear separation of *B. franklini* from other species within the subgenus. They furthermore indicated that the male genitalia of *B. franklini* are markedly different from those of *B. occidentalis* and advocated retention of specific status for *franklini*, they concluded (Plowright & Stephen 1980: 479): “The origin of *B. franklini* is mysterious. The results from the present study give no indication that it is closely related to any of other nearctic representatives of its subgenus.”

In a recent study, Scholl et al. (1990) investigated the genetic relationships of Nearctic and Palaearctic representatives of the subgenus *Bombus* s.str. by enzyme electrophoretic data with special reference to *B. moderatus* Cresson, another problematical taxon in this group. *Bombus franklini* unfortunately could not be included in this analysis because it was not available at that time. We now have been able to collect *B. franklini* in northern California and in Oregon and we have compared it electrophoretically with the previously studied representatives of the subgenus *Bombus* s.str.

## MATERIAL AND METHODS

*Specimens Analyzed.*—*Bombus franklini*: CALIFORNIA. SISKIYOU Co.: Hilt, 29 Jul 1989, 12 workers; 6.4 km (4 mi) E of Yreka, 29 Jul 1989, 2 workers. OREGON. JACKSON Co.: Ashland, 29 May 1990, 4 queens and 1 worker; Ruch, 29 May 1990, 12 workers; Central Point, 30 May 1990, 2 workers; Gold Hill, 30 May 1990, 12 workers.

Frozen homogenates of previously studied material [*B. affinis* Cresson, *B. cryptarum* (Fabr.), *B. hypocrita* Pérez, *B. lucorum* (L.), *B. moderatus*, *B. occidentalis*, *B. terrestris* auct., *B. terricola* Kirby and *B. sporadicus* Nylander] (Scholl et al. 1990) and additional specimens, including one queen, nine workers and one male of *B. occidentalis* from the same localities where *B. franklini* was collected and 10 queens, 38 workers and two males of *B. occidentalis* from eight other localities in northern California have been used for electrophoretic comparison. This material is summarized in Table 1. Californian *B. occidentalis* included the nominate subspecies and *B. o. nigroscutatus* Franklin along with their intergrades, these are not listed separately because the electrophoretic data did not indicate any difference.

*Electrophoresis.*—We have used the same methods (vertical starch-gel electrophoresis) and enzymes (21 loci) as Scholl et al. (1990). These enzymes are: Aconitase, 2 loci: Acon-1 and Acon-2; Arginine kinase, Apk; Hydroxybutyric dehydrogenase, Bdh; Esterase, Est-1;  $\alpha$ -Glycerophosphate dehydrogenase, 2 loci:  $\alpha$ -Gpd-2 and  $\alpha$ -Gpd-3; Glutamic-oxaloacetic transaminase, Got-2; Glutamic-pyruvic transaminase, Gpt; Hexokinase, 2 loci: Hk-1 and Hk-3; Isocitrate dehydrogenase, 2 loci: Idh (NAD) and Idh (NADP); Leucine aminopeptidase, Lap; Malate dehydrogenase, 2 loci: Mdh-1 and Mdh-2; Malic enzyme, Mod; Peptidase, Pep; Phosphoglucose isomerase, Pgi; Phosphoglucomutase, Pgm; Superoxide dismutase, Sod (for details see Scholl et al. 1990). A phenogram of the genetic relationships of the species investigated was constructed by average linkage cluster analysis (UPGMA) (Nei 1987) using Nei's (1972) standard coefficient of genetic identity (I).

## RESULTS AND DISCUSSION

Ten of the 21 loci scored were found invariant in all species surveyed. These loci are: Acon-2, Apk, Bdh,  $\alpha$ -Gpd-1,  $\alpha$ -Gpd-2, Idh (NADP), Lap, Mdh-2, Mod, and Sod. Eleven loci showed interspecific variation. The zymograms observed are schematically shown in Fig. 1, where the designation of electromorphs is based on mobilities (in mm) relative to the electromorph of *B. lucorum* (= index 100), as in previous electrophoretic studies on bumble bees (e.g., Scholl & Obrecht 1983, Scholl et al. 1990).

*Bombus franklini* was monomorphic in all loci scored, except Pep, where one worker was heterozygous. Minor polymorphisms were observed in some loci of other species. These are: Acon-1 in *B. lucorum*, *B. terrestris* and *B. sporadicus*; Est-1 in *B. lucorum* and *B. terricola*, Got-2 in *B. cryptarum*, *B. magnus*, *B. lucorum*, *B. occidentalis*, *B. terrestris* and *B. sporadicus*; Hk-1 in *B. occidentalis*; Mdh-1 in *B. terrestris*. The level of polymorphism, however, was usually very low ( $H < 0.05$ ), as also observed previously in other bumble bee species (Obrecht & Scholl 1981, Pamilo et al. 1984), except in Got-2 of *B. occidentalis*, where the

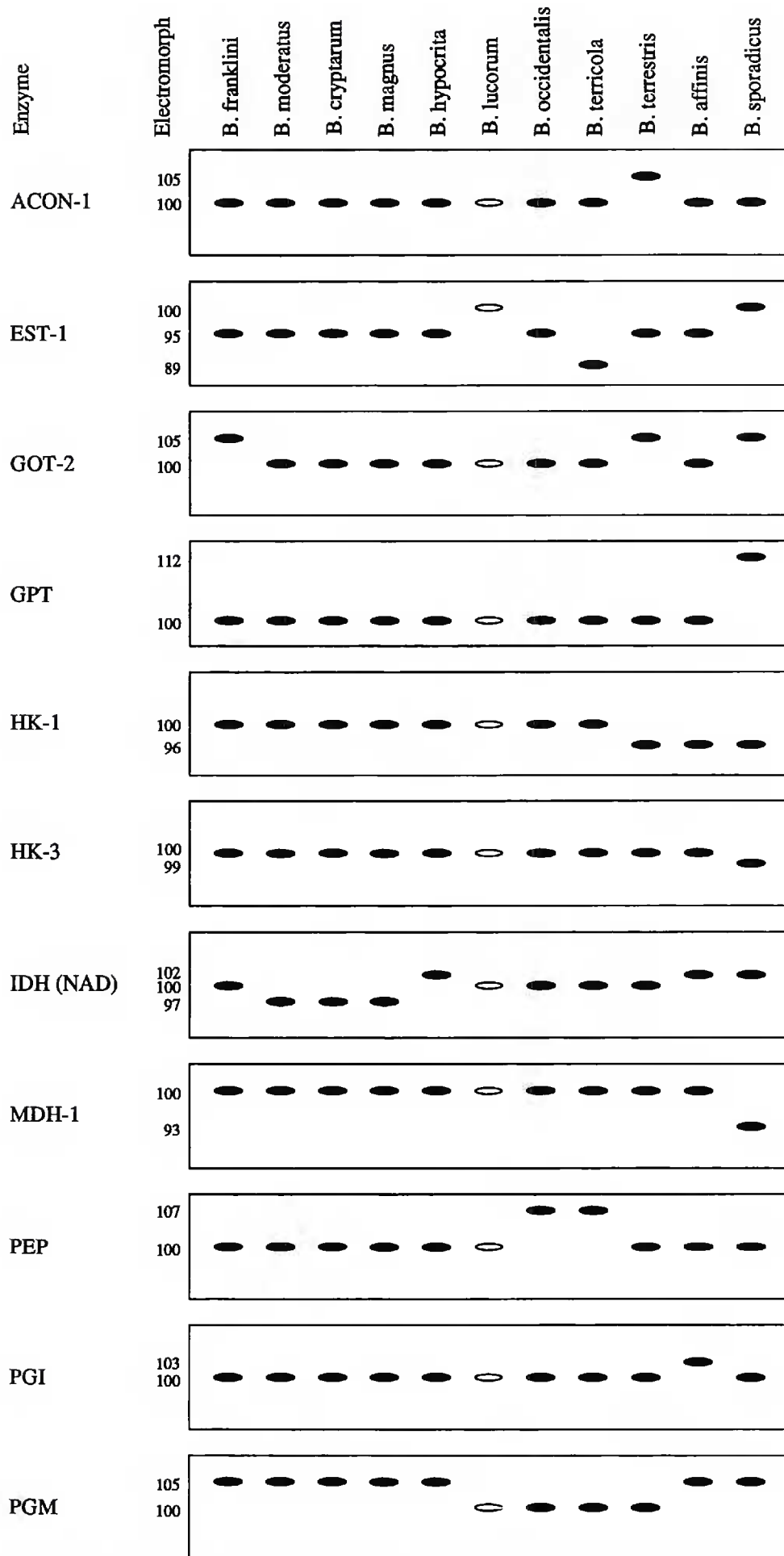


Figure 1. Schematic illustration of enzyme phenotypes in *Bombus* s.str. species. (Note: *B. lucorum* is the reference, assigned mobility index = 100 for each enzyme.)

frequency of a minor allele Got-2<sup>105</sup> ranged between 0.05 and 0.25 at three sampling sites in Alberta, Calgary, Barrier Lake and Fortress Mountain respectively, while allele Got-2<sup>100</sup> was fixed in samples from other areas (Table 1).

We have not found an electromorph that is unique to *B. franklini*. However,

Table 1. Specimens analyzed and origin of material.

		California	Oregon	Alaska	British Columbia	Alberta	Ontario	Japan	N-Europe	E-Europe	C-Europe	S-Europe	Total
<i>B. franklini</i>	♀	14	31	—	—	—	—	—	—	—	—	—	45
( <i>n</i> = 45)													
<i>B. moderatus</i>	♀	—	—	—	—	8	—	—	—	—	—	—	8
( <i>n</i> = 9)	♂	—	—	—	—	1	—	—	—	—	—	—	1
<i>B. cryptarum</i>	♀	—	—	—	—	—	—	—	2	—	13	—	15
( <i>n</i> = 22)	♂	—	—	—	—	—	—	—	5	—	2	—	7
<i>B. magnus</i>	♀	—	—	—	—	—	—	—	5	—	11	—	16
( <i>n</i> = 17)	♂	—	—	—	—	—	—	—	—	—	1	—	1
<i>B. hypocrita</i>	♀	—	—	—	—	—	—	8	—	—	—	—	8
( <i>n</i> = 8)													
<i>B. lucorum</i>	♀	—	—	—	—	—	—	—	2	2	41	4	49
( <i>n</i> = 57)	♂	—	—	—	—	—	—	—	6	—	2	—	8
<i>B. occidentalis</i>	♀	54	2	8	1	36	—	—	—	—	—	—	101
( <i>n</i> = 110)	♂	2	—	—	—	7	—	—	—	—	—	—	9
<i>B. terricola</i>	♀	—	—	—	—	4	11	—	—	—	—	—	15
( <i>n</i> = 18)	♂	—	—	—	—	—	3	—	—	—	—	—	3
<i>B. terrestris</i>	♀	—	—	—	—	—	—	—	—	3	29	8	40
( <i>n</i> = 40)													
<i>B. affinis</i>	♀	—	—	—	—	—	11	—	—	—	—	—	11
( <i>n</i> = 11)													
<i>B. sporadicus</i>	♀	—	—	—	—	—	—	—	15	—	—	—	15
( <i>n</i> = 15)													

N-Europe = Finland, Norway, Scotland, England; E-Europe = Hungary; C-Europe = Switzerland, France, Belgium; S-Europe = Spain, Italy.

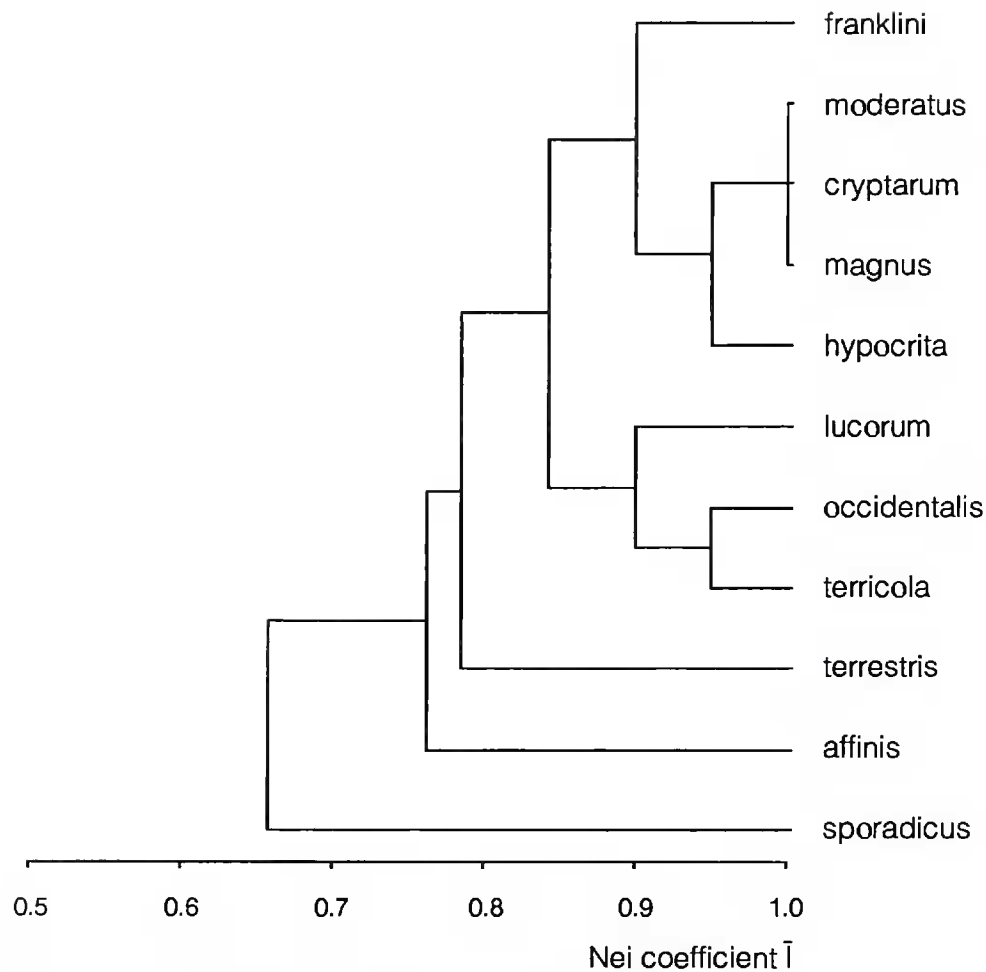


Figure 2. Phenogram showing the genetic relationship between *B. franklini* and other taxa of the subgenus *Bombus* s.str., as revealed by the electrophoretic data.

it is the electrophoretic pattern of enzymes that is unique to *B. franklini* (Fig. 1). Thus, *B. franklini* differs from *B. moderatus* in GOT-2 and IDH (NAD); *B. terrestris* is identical with *B. franklini* in GOT-2 and IDH (NAD), but these species differ in ACON-1, HK-1 and PGM, etc. *Bombus franklini* differs from *B. occidentalis* in GOT-2, PEP and PGM; the Got-2 locus, however, was found weakly polymorphic in three *B. occidentalis* samples from Southern Alberta, as mentioned above. In our material from California and Oregon, there was always a clear separation on the basis of these three enzymes and the electrophoretic data did not provide any evidence of intergradation of *B. franklini* and *B. occidentalis*.

The genetic relationships of *B. franklini* and other Nearctic and Palaearctic representatives of its subgenus, as revealed by the enzyme electrophoretic studies, are presented in Fig. 2 as a phenogram that is based on a similarity matrix (Nei coefficient  $I$ ) calculated in pairwise species comparisons from the 21 loci surveyed. According to these data, *B. franklini* is close to two species groups that comprise: (a) the European *B. cryptarum* and *B. magnus*, the North American *B. moderatus* and the Japanese *B. hypocrita*; and (b) the European *B. lucorum* and the North American *B. terricola* and *B. occidentalis*, while the European *B. terrestris* and *B. sporadicus* and the North American *B. affinis* are more distant. This new information adds to, but does not alter the basic genetic relationships among species of the subgenus *Bombus* as determined by Scholl et al. (1990).

The narrow endemism of *B. franklini* is intriguing. As Thorp (1970) pointed out, all recent records have been taken within a 60 mile radius of Grants Pass, Oregon. *Bombus franklini* is keyed out from sympatric *B. o. occidentalis* by its

coat color. But Stephen (1957) found that separation is often difficult. The northwestern coast is an area where several bumble bee species, including *B. occidentalis*, show gradation from one color form to another resulting in color convergence toward local Müllerian mimicry groups (Plowright & Owen 1980, Thorp et al. 1983). However, *B. occidentalis* females do not have yellow anterolaterally on the scutum, extending back beyond the tegulae, and *B. franklini* females are uniform in color throughout the known range (Thorp et al. 1983). One might speculate that *B. franklini* has in fact a more widespread distribution, but becomes hidden within the color variation of *B. occidentalis*. The electromorphetic data presented here provide an opportunity to test this hypothesis.

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